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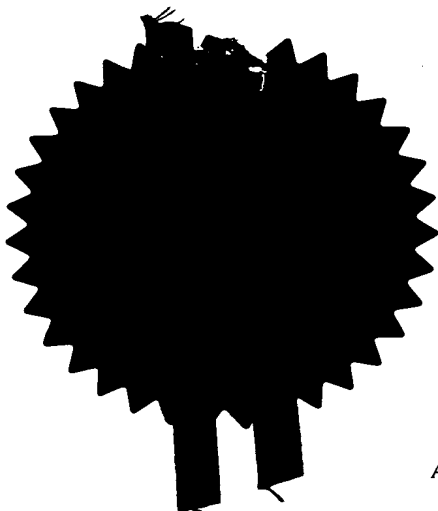
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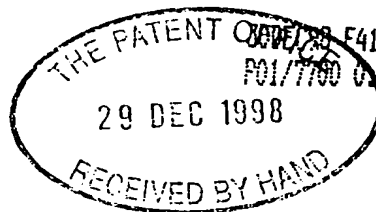


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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)



The Patent Office

Cardiff Road
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2. Your reference PJF/DSH/10025GB

2. Patent application number
(The Patent Office will fill in this part)

9828765.9

29 DEC 1998

3. Full name, address and postcode of the or of each applicant (underline all surnames)

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Patents ADP number (if you know it)

6910301003

7361132001

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

A Method of Testing A Cell Sample

5. Name of your agent (if you have one)

ELKINGTON AND FIFE

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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Patents ADP number (if you know it)

67004

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)

Date of Filing
(day/month/year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of Filing
(day/month/year)

8. Is a statement of inventorship of right to grant of a patent required in support of this request? (Answer "Yes" if:

No

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
- c) any named applicant is a corporate body.

See note (d))

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	0
Description	7
Claim(s)	2
Abstract	0
Drawing(s)	3

Ph

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination (Patents Form 10/77)

Any other documents
(please specify)

11. I/We request the grant of a patent on the basis of this application.

Signature

Nick Gmk

Date

29 December 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr Peter J Finnie
0171 405 3505

A METHOD OF TESTING A CELL SAMPLE

All types of blood cells occasionally agglutinate, one category of which is red cell agglutination that frequently heralds a serious haemolytic disease. It may indicate an underlying malignancy such as non-Hodgkin's lymphoma, Hodgkin's disease, acute lymphocytic leukemia, carcinoma, thymoma and ovarian tumours. It occurs in blood group incompatibility as in haemolytic disease of the newborn, and mis-matched blood transfusions; also in paroxysmal nocturnal haemoglobinuria and hypogammaglobulinaemia; in some collagen diseases such as disseminated lupus erythematosus, rheumatoid arthritis, ulcerative colitis and hepatitis; in some infections such as viral and Mycoplasma pneumonia, cytomegalovirus, tuberculosis and infectious mononucleosis, and as a toxic reaction to some drugs such as L-dopa. As the presence of intra or extra vascular haemolysis in these diseases carries at least a 10% mortality, the identification of red cell agglutination is useful for the early diagnosis and for monitoring the response to treatment.

Traditionally, agglutination is detected by visually observing and counting the number of clumped aggregates of cells. Whilst automated cell counters have supplanted all manual routine haematology they cannot detect agglutination sufficiently accurately to avoid manual verification. Indeed, existing automated cell counters erroneously measure agglutinated clumps of cells as one large cell producing an inaccurate mean cell volume and cell count and compound indices derived from them. An abnormally high mean corpuscular volume (MCV) or an abnormally elevated mean corpuscular haemoglobin concentration (MCHC) displayed by commercial haematology

autoanalysers alerts the technician to the possibility of the presence of agglutination. However, these indices are inadequate indicators of agglutination because they are not specific, moreover agglutination must rise to high levels before the indices exceed the normal limits. An elevated MCHC is also produced by red cell fragmentation, lymphocytosis, hyperglycemia and haemoglobinaemia and therefore requires manual inspection and further testing to establish the diagnosis.

According to the present invention, a method of detecting agglutination in a sample of cells comprises the steps of inducing cells to change at least one of their properties so as to separate agglutinated cells and detecting the resultant alteration in the cell population.

Preferably, the property change is that of the shape of the cells. More preferably, the cell sample is subject to an alteration in environment to cause the cells to sphere. In a preferred example, the alteration in the environment is a change in osmolality of a liquid medium in which the cells are suspended, preferably by the addition of water.

Preferably, alterations in the cell population are detected by passing one or more aliquots of the cell sample through a sensor which is adapted to count the number of cells passing through the sensor. More preferably, the sample is fed continuously into a solution the osmolality of which is changed continuously to produce a continuous series of aliquots of cells which are passed through the sensor.

Preferably, the method further comprises the step of pre-treating the sample of cells

to induce, or at least attempt to induce, agglutination. In one preferred use of the invention, a cell sample of unknown antigenicity from one source is mixed with antibodies from a different source. The antibodies may be manufactured or come from whole blood, plasma or typically serum. In a further step, the putative antigen-antibody mixture may be tested at different temperatures to reveal heat sensitive agglutination.

The present invention measures agglutination using a process which is also capable of testing how tightly agglutinated cells are bonded by measuring how much force is required to separate them. This property depends upon antibodies interacting with the complement system. Agglutination of red blood cells is a function of the type and number of antigen combining sites on the surface of the cells, which bind with complementary IgC antibody molecules. The strength of agglutination is a function of the proximity of the binding sites on the cell surface. By placing a whole blood sample into a typically 1:10,000 suspension, and causing red cells which are approximately bi-concave discs to sphere, the effective surface area available for bonding diminishes. Sphering a cell increases the space between antigen binding sites and increases the mean distance across which bonding occurs. As the surface area available for bonding between cells decreases as cells sphere they lose bonding strength, thus allowing clumped cells to separate. By recording the inducing pressure and the number of cells (or quantities related to it) as they change with respect to the inducing pressure, agglutination can be detected, quantified and monitored. Cells which have agglutinated, when tested by this method, separate and thereby increase the cell count in a characteristic fashion. In a further step the sample is subject to

mechanical agitation which tends to promote agglutination in normally shaped cells capable of agglutination but promotes separation of spherically shaped cells.

An example of the present invention will now be described in detail with reference to the accompanying drawings, in which:

Figure 1 is a graph showing changes in red cell count and cell volume for a normal patient whose normal non-agglutinable biconcave red blood cells suspended in a liquid medium are exposed to a progressive reduction in ambient osmolality;

Figure 2 is a graph showing the results for a first abnormal patient having agglutinated blood cells;

Figure 3 is a graph showing the results for a second abnormal patient having agglutinated blood cells;

Figure 4 is a graph showing the formation of cell fragments;

Figure 5 is a screen dump of a set of results from an automatic blood cell analyzer of the type described in detail in International patent application PCT/GB96/24601, for a patient having agglutinated blood cells; and,

Figures 6A to 6C show results from another patient.

The method of the present invention is exceptionally useful in conjunction with the methods and apparatus described in the applicants' earlier filed International patent applications, namely, PCT/GB 96/24601, PCT/GB 96/03256, and PCT/GB 96/03259 and enhances the general utility of the tests described therein.

The preferred method consists of counting the cells as they pass through an aperture.

The instrument may be configured with a mixing chamber into which saline, cells and diluent are injected, in which case the number of cells passing through the aperture at every osmolality does not vary. When only two streams are injected into the mixing chamber, diluent and a saline suspension into which the cells have been previously introduced, the number of cells passing through the aperture is fixed at a level that is directly proportional to the osmotic gradient. The results for a normal patient are shown in Figure 1. In Figures 1 to 4 and 6A the top plot represents the red cell count, and the bottom plot represents volume. Deviation from the predetermined straight line of cell count against osmolality (see Figures 2 to 4) can only occur if additional particles appear, or are stimulated by the ambient change in pressure. Two types of particle may be generated: disrupted agglutinated cells (DACs) or cell fragments, and they are both distinguishable by their size or other features. Fragments vary inversely with osmolality while DACs vary directly with sphering, thus the range of pressures in the interval between P_{\max} and P_0 , where P_{\max} is the point at which the rate of fluid flow into the cell reaches a maximum and P_0 is the equilibrium point, converts agglutinated cells into DACs and the count increases in this interval when agglutinated clumps are disrupted (see Figures 2 and 3). As shown in Figure 4, fragments typically begin to appear around P_0 becoming increasingly frequent thereafter, whereas DACs do not appear after P_0 . Other characteristics of the change in the suspended cell population may equally be used to detect and differentiate the type of particle, as may the addition of dyes, complement, antisera or other agents.

When cells agglutinate or are made to agglutinate, the cell count will fall; then, as the

cells sphere, the cell count increases with each aggregate tending to separate into its component parts in inverse proportion to the strength of the agglutination.

Fragments and DACs can also be segregated by size. Fragments are quite small
5 between 10 and 30 fl in volume whereas DACs are at least three times the size,
generally between 60-110 fl. In addition, the isotonic MCV is normal or reduced in
the presence of fragments while the MCV is elevated with agglutination. As the range
of normal MCV is so large it can hide much agglutination. Sample ageing and the
application of mechanical, ultrasound or other stress increases the count of intact cells
10 if the sample was agglutinating and decreases the number of intact cells if the sample
is fragmenting.

The method may be embodied into an instrument for the automated recognition of
blood groups and cell types by the induction and detection of agglutination by mixing
15 anti-bodies and cells in the mixing chamber and detecting agglutination, or the lack
of it, at the sensor aperture by counting, thus eliminating manual blood grouping and
cross-matching. The results generated by such an instrument described in International
patent application PCT/GB96/24601 are shown in Figure 5.

20 Figures 6A to 6C show the results for another patient. The sudden increase in cell
count at sphering is shown clearly in Figure 6A, and the increased sphericity index
appears as a fat cell in Figure 6B. Figure 6C shows the increase in variance of the
red cell frequency distribution due to agglutinated clumps of cells. The standard
deviations are about twice the normal.

The present invention is particularly useful in the early detection of agglutination, hence the early detection and subsequent treatment of haemolytic diseases, and enhanced possibility of recognizing the underlying pathology. It is also possible to quantify the strength of cell agglutination from the extent to which separation is achieved and the ease with which it is achieved. As the unagglutinated cell concentration is known any reduction in the isotonic count represents agglutination. As the cell suspension is exposed to the sphering gradient, the original count will be restored at higher osmolalities and in proportion to the strength of the agglutination. Finally, the method provides for the automatic identification of blood groups and cell types by inducing cells to agglutinate and subsequently testing them using the method.

CLAIMS

1. A method of detecting agglutination in a sample of cells, comprising the steps of inducing the cells to change at least one of their properties so as to separate agglutinated cells and detecting the resultant alteration in the cell population.

2. A method according to claim 1, comprising the step of measuring the force required to separate agglutinated cells.

3. A method according to claim 1 or 2, in which the property changed is that of the shape of the cells.

4. A method according to any preceding claim, in which the cell sample is subject to an alteration to cause the cells to sphere.

5. A method according to claim 4, in which the alteration is a change in osmolality of a liquid medium in which the cells are suspended.

6. A method according to any preceding claim, in which alterations in the cell population are detected by passing one or more aliquots of the cell sample through a sensor which is adapted to count the number of cells passing through the sensor.

7. A method according to claim 6, in which the sample is fed continuously into a solution the osmolality of which is changed continuously to produce a continuous

series of aliquots of cells which are passed through the sensor.

8. A method according to any preceding claim, further comprising the step of pretreating the sample of cells to induce, or at least attempt to induce, agglutination.

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9. A method according to any preceding claim, in which the cell sample is obtained from a source of whole blood.

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10. A method according to claim 8 or 9, in which the sample of cells are treated by cross matching with antibodies from a different source.

11. A method according to claim 10, in which the antibodies from the different source are manufactured, or come from whole blood, plasma or serum.

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12. A method according to any of claims 8 to 11, in which the sample of cells is pre-treated by exposure to heat.

13. A method according to any of claims 8 to 11, in which the sample of cells is pre-treated by cooling the sample.

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14. A method of detecting agglutination in a sample of cells substantially as shown in and/or described with reference to any of Figures 2 to 5 of the accompanying drawings.

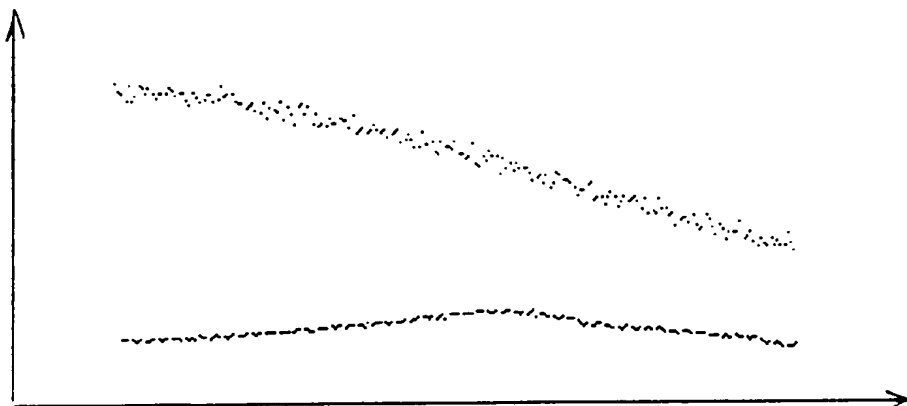


FIG 1

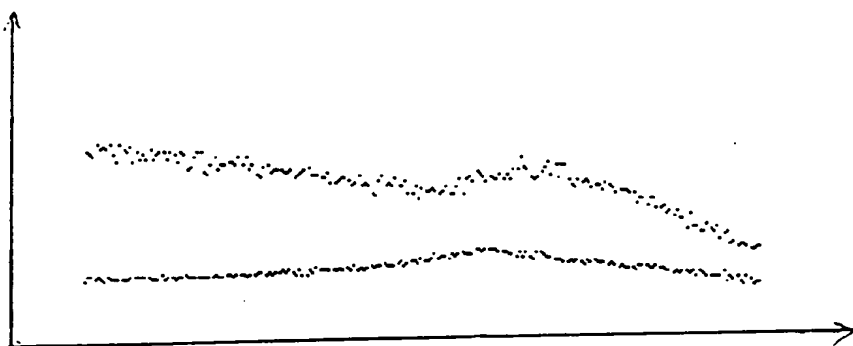


FIG 2

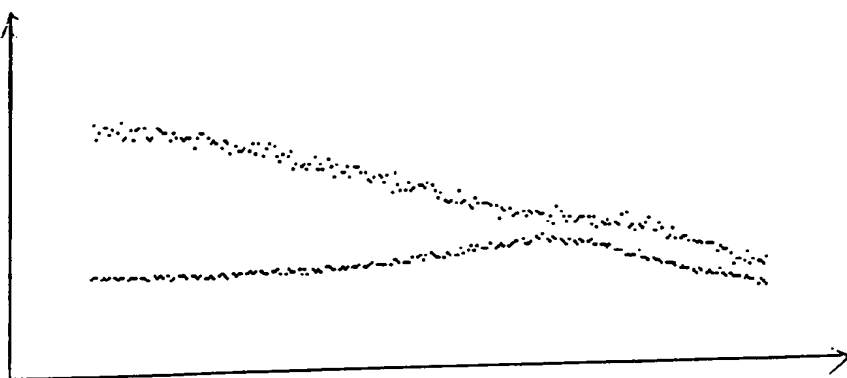


FIG 3

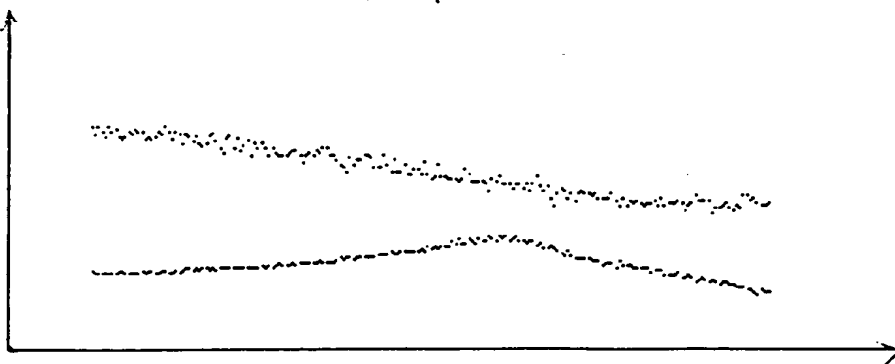


FIG 4

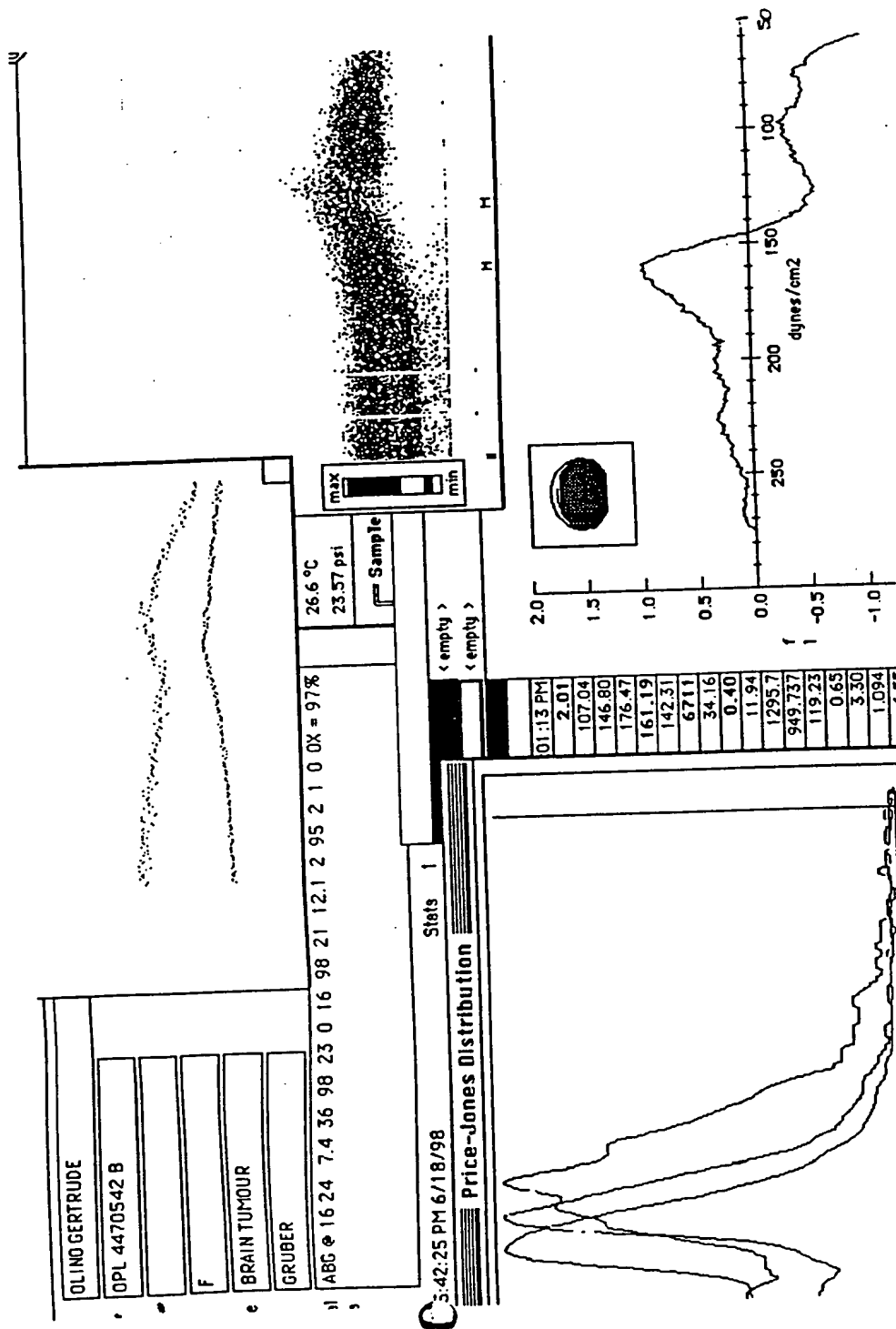


FIG 5

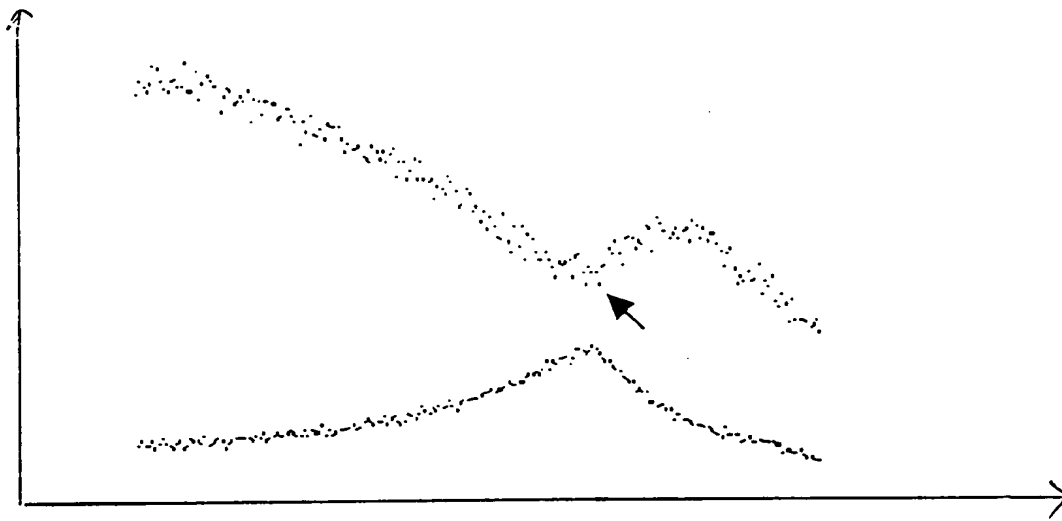
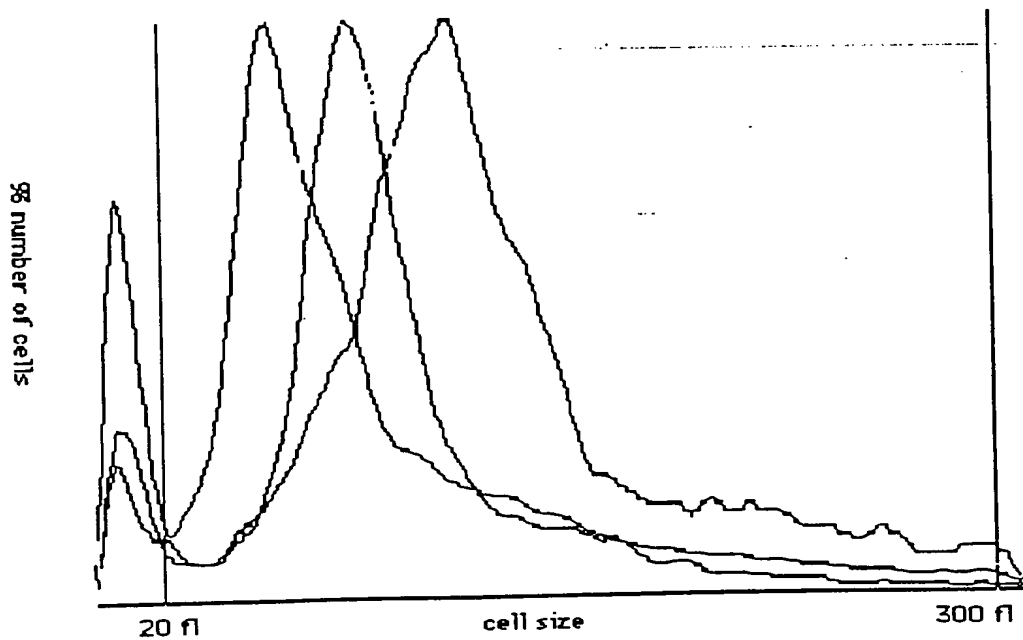


FIG 6A



FIG 6B







L: 20	R:	Color	mean	SD	cv	skew	kurtosis	n(k)
Isotonic curve			79.0	45.5	58	1.5	-2.8	43.3
Spherical curve			109.6	44.0	40	1.0	0.2	6.0
Ghost			83.8	33.2	40	1.6	3.6	7.4
User			93.4	44.9	48	1.3	-2.7	28.8

FIG 6C